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THE MANUFACTURE AND STUDY OF HEMOGLOBIN - SALINE SOLUTION.(U)
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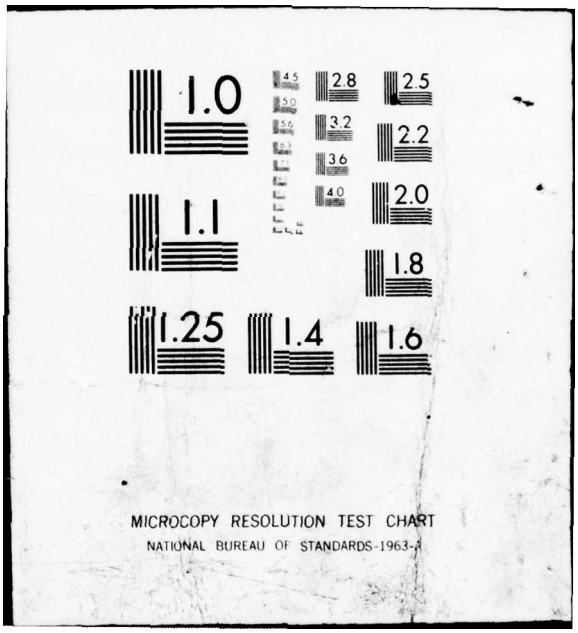
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A PROPOSAL SUBMITTED BY

MICHAEL REESE HOSPITAL & MEDICAL CENTER

2900 SOUTH ELLIS AVENUE, CHICAGO, ILLINOIS 60616

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I. WORK COMPLETED

1. OXYGEN TRANSPORT FROM TWO HEMOGLOBIN COMPARTMENTS

INTRODUCTION

Two compartments for hemoglobin exist during the exchange transfusion of a primate with hemoglobin-saline solution. One is the endogenous hemoglobin within the red blood cells, and the other is the exogenous hemoglobin in the extra-erythrocytic space. Because of differences in oxygen affinity and in physical environment, the loading and unloading of oxygen may not occur in a parallel fashion in the two compartments.

The purpose of the current study was to evaluate loading and unloading of oxygen, in each compartment, during exchange transfusion of primates.

MATERIALS AND METHODS

Five adult baboons, weighing from 20.2 to 29.1 kg were the test animals. On the morning of each study, the baboon was tranquilized within its cage with an intramuscular injection of 0.8 mg/kg of phencyclidine hydrochloride piperazine. Under local anesthesia, plastic catheters were inserted in bilateral femoral arteries and one femoral vein. They were positioned in the inferior vena cava and the abdominal aorta. A Swan-Ganz thermistor-tipped catheter was flow-directed under

fluoroscopy, into the pulmonary arterial bed. Statham pressure transducers were connected to the pulmonary artery and abdominal aorta to monitor aortic, pulmonary arterial, and wedge pressures. The remaining pair of catheters was used for the exchange transfusion. Cardiac output was determined by thermal dilution, using a Columbus Instrument Computer. A lead II EKG was obtained with needle electrodes. The signals from all transducers were recorded on a Brush multichannel oscillograph. A temperature probe was positioned in the abdominal cavity through a small incision in the abdominal wall. The trachea was intubated and the baboon was paralyzed by frequent I.V. injections of d-tubocurarine. The baboon was mechanically ventilated with room air, in the prone position, with axillary supports. During the base line period the tidal volume and respiratory rate were adjusted to produce an arterial pCO_2 between 33 and 47 mm Hg. These ventilator settings were held constant throughout each study. The body temperature was stabilized by raising the ambient temperature.

The experimental protocol consisted of our standard exchange transfusion with hemoglobin-saline solution. Blood samples and hemodynamic measurements were obtained during the control period, and at hematocrits of 30, 20, 10, and 5. Hemodynamic variables were heart rate, aortic pressure, pulmonary arterial pressure, wedge pressure, and cardiac output. Arterial and mixed venous blood samples were obtained anerobically. Aliquots were centrifuged to provide anerobic samples of arterial and mixed venous plasma. Arterial and mixed venous

whole blood gases were determined using an IL 313 blood gas analyzer. Oxygen content was obtained using a Lexington Instrument fuel cell. Oxygen contents were measured on whole blood and plasma aliquots from the arterial and venous samples. Hematocrit was determined in a standard manner with micro-hematocrit tubes and a centrifuge. Whole blood and plasma capacity were estimated by equilibrating samples with room air, and measuring oxygen contents. Red cell contents and capacities were calculated from the whole blood and plasma values, using the balance equations. Fractional saturations were calculated from contents and capacities. Hemoglobin concentrations were calculated from capacities, assuming standard binding coefficients for each type of hemoglobin. Oxygen consumption was calculated from the cardiac output and the whole blood arterial-venous oxygen content difference.

RESULTS

Table I contains the hemodynamic and consumption data. The mean arterial pressure showed a transient increase at the start of the transfusion, followed by a progressive fall in blood pressure. The mean pulmonary arterial pressure remained constant until the lowest hematocrit, when it began to rise. The pulmonary wedge pressure did not change during the exchange transfusion. The cardiac output, body temperature, and oxygen consumption were constant during the exchange transfusion. The heart rate decreased during the early

phases of the transfusion. As the exchange continued, the heart rate increased.

Table II contains the blood gas data. The pH dropped slightly during the transfusion. No changes were observed in the pCO_2, or in the arterial PO_2. An obvious decrease in venous PO_2 occurred during the study.

Tables III and IV show the oxygenation data for the two compartments. The hemoglobin concentration is expressed as grams hemoglobin per 100 cc of whole blood. There was a progressive decrease in rbc hemoglobin, with a concomitant increase in plasma hemoglobin. The oxygen content is expressed as cc O_2, per gram hemoglobin of that compartment. The arterial contents of both compartments did not change. The venous contents of the red cells fell markedly below a hematocrit of 30. The venous plasma contents exhibited a slight drop below a hematocrit of 20. The fractional saturation data was parallel to the contents. The "extraction ratio" is defined, for each compartment, as the A-V O_2 difference divided by the arterial content. The extraction ratio for each compartment, is a function of the oxygen consumed, and the interchange of oxygen between compartments. The red cell extraction ratio rose markedly below a hematocrit of 30. The plasma extraction ratio showed a slight increase at the lowest hematocrits.

TABLE I

	HEMATOCRIT				
	40	30	20	10	5
MEAN ARTERIAL PRESSURE	121*	143	94	76	65
(mmHg)	±11**	14	13	1	--
MEAN PULMONARY ARTERIAL	12	17	14	15	25
PRESSURE (mmHg)	3	1	4	14	--
PULMONARY WEDGE PRESSURE	2	1.5	1	-1	--
(mmHg)	2	2	--	--	--
CARDIAC OUTPUT	3.1	2.3	3.8	3.3	2.6
(L/min)	1.	0.8	0.4	0.1	1.8
HEART RATE	125	118	158	142	175
(beats/min)	26	31	25	25	--
TEMPERATURE	35.6	34.1	36.7	36.2	35.9
(° C)	2.3	1.	1.3	2.	1.6
OXYGEN CONSUMPTION	4.3	2.8	5.1	4.0	2.9
(cc/min - kg)	2.1	--	1.5	2.	2.

* Mean

** Standard Deviation

TABLE II

BLOOD GASES

	Hematocrit				
	40	30	20	10	5
ARTERIAL					
pH	7.41*	7.34	7.33	7.25	7.14
	±.04**	±.06	±.12	±.12	±.07
pCO_2	29.00	35.00	20.00	37.00	29.00
	±8.00	±5.00	±5.00	±10.00	±2.00
pO_2	110.00	118.00	102.00	104.00	94.00
	±31.00	±1.00	±35.00	±30.00	±37.00
VENOUS					
pH	7.38	7.39	7.30	7.20	7.10
	±.04	---	±.17	±.11	±.08
pCO_2	39.00	39.00	46.00	42.00	36.00
	±6.00	---	±16.00	±9.00	±2.00
pO_2	55.00	52.00	36.00	29.00	24.00
	±11.00	---	±19.00	±13.00	±4.00

* Mean

** Standard Deviation

TABLE III

	HEMATOCRIT				
	40	30	20	10	5
HEMOGLOBIN					
(gm/100 cc)					
Plasma	0	0.8	1.3	1.8	2.4
	--	±.2	±.1	±.1	±.1
RBC	10.1*	7.5	5.2	2.6	1.2
	±1.7**	±.06	±.1	±.6	±.1
OXYGEN CONTENT					
(cc O_2/gmHb)					
Arterial RBC	1.15	1.24	1.20	1.17	1.28
	± .13	±.18	±.05	±.19	±.15
Arterial Plasma	--	0.98	1.20	1.22	1.32
	--	±.06	±.11	±.01	±.02
Venous RBC	0.88	0.83	0.49	0.29	0.04
	±.04	--	±.34	±.34	±.06
Venous Plasma	--	.78	1.31	1.00	.96
	--	--	± .01	± .08	± .06

* Mean

** Standard Deviation

TABLE IV

	Hematocrit				
	40	30	20	10	5
Fractional Saturation					
Arterial RBC	.91*	.92	.91	.88	.94
	±.08**	±.13	±.04	±.13	±.08
Arterial Plasma	--	.73	.88	.92	.97
	--	±.03	±.06	0	0
Venous RBC	.65	.62	.37	.21	.03
	±.03	--	±.25	±.26	±.04
Venous Plasma	--	.58	.83	.76	.72
	--	--	±.01	±.06	±.04
Extraction Ratio					
RBC	.26	.25	.60	.72	.97
	±.06	--	±.27	±.34	±.04
Plasma	--	.18	.05	.18	.26
	--	--	±.06	±.07	±.04

* Mean

** Standard Deviation

DISCUSSION

The data in Tables III and IV shows that both forms of hemoglobin are capable of loading oxygen in the pulmonary circulation. The quantitative differences are too small to be discerned by our data. Major differences occur in the unloading, as shown by the venous data. Red cell hemoglobin loses its oxygen more rapidly and completely than the plasma hemoglobin. In the 10% to 20% hematocrit range, which is of clinical interest, the red cells have lost 60% to 70% of their oxygen, while the plasma hemoglobin have lost 5% to 15% of theirs. At the lowest hematocrits, the red cells have lost 97% of their oxygen, while the plasma hemoglobins have only lost 26% of theirs.

The P_{50} of normal baboon hemoglobin is in the 26-30 mmHg range. The P_{50} of the hemoglobin solution varies between 12-16 mm Hg. Most experimental and theoretical studies show that the transit time through the exchange vessels is long enough for chemical equilibrium to occur between hemoglobin and oxygen. Hence, our results are consistent with the difference in the oxygen dissociation curves of the two species of hemoglobin.

The amount of oxygen released by hemoglobin-saline solution is a small fraction of the total, until the hematocrit falls to extremely low levels. Evidence suggests that a major factor is the P_{50} of the hemoglobin solution. An increase in P_{50} to about 28 mm Hg should improve the unloading from the plasma compartment, in the presence of red cell hemoglobin.

2. INTRAVASCULAR PERSISTENCE OF 2,3-DPG

INTRODUCTION

The low P_{50} of stroma-free hemoglobin solution is due to several reasons, including the "stripped" nature of the hemoglobin molecule. Normal ligands such as 2,3-DPG or ATP are absent, because the hemoglobin solution is prepared from outdated blood. One direct approach to normalization of P50 would be to include exogenous 2,3-DPG during the administration of SFH. The purpose of this pilot study was to evaluate intravascular persistence of an infusion of 2,3-DPG.

MATERIALS AND METHODS

2,3-DPG was obtained from Sigma Chemicals as the pentacyclohexylammonium salt. The free acid was obtained by conversion with Dowex-50w. DPG levels were assayed using an ultra-violet technique (Sigma 35-UV). An infusion solution was prepared containing 30 micro-moles/cc of DPG.

A 19.1 kg male baboon was prepared in the usual fashion with catheters in the abdominal aorta and inferior vena cava. The urinary bladder was catheterized. A continuous infusion of 2,3-DPG was started and maintained at a 3 milli-mole/hour rate for two hours. Heart rate, arterial and central venous pressures, respiratory rate, and hematocrit

were monitored during the infusion. Samples of whole blood, plasma, and urine were analyzed for DPG during the control period, and after one and two hours of infusion.

RESULTS

Table I contains the baseline hemodynamic values. These did not change during the two hours of infusion.

Table II contains the DPG concentrations during the control period, and after one and two hours of infusion. No significant changes in whole blood DPG concentrations were seen. The plasma levels were below the limits of sensitivity of the assay. No DPG was seen in the urine during the control period. Significant amounts of DPG were present in the urine after the start of infusion.

DISCUSSION

We estimate that urinary loss accounted for about 10% of the infused DPG. If the DPG was distributed uniformly in the total extracellular space, the resulting plasma concentration would be below the limits of the assay. In addition, there is evidence that DPG can be converted by plasma enzymes, both in vivo and in vitro.

This pilot study showed that there is no significant plasma

persistence to infused DPG. It is possible that the weak chemical bond between DPG and de-oxyhemoglobin would protect the DPG from the mechanisms of removal. However, the DPG would be unprotected when the hemoglobin became oxygenated. We conclude that normalization of the hemoglobin P_{50} cannot be accomplished using free DPG.

TABLE I

VITAL SIGNS

	Control Period
Hematocrit	44
Respiratory Rate	33/min
Heart Rate	125/min
Mean Arterial Pressure	135 mm Hg
Systolic Arterial Pressure	165 mm Hg
Diastolic Arterial Pressure	115 mm Hg
Central Venous Pressure	9 mm Hg

TABLE II

2,3-DPC LEVELS

	Control Period	One Hour Infusion	Two Hour Infusion
Whole Blood	1.73*	2.23	2.08
Plasma	0	0.15 (N.S.)	0.23 (N.S.)
Urine	0.15 (N.S.)	3.50	2.54

*Concentration expressed as micro-moles/cc

3. NEURONAL FUNCTION DURING INFUSION OF HEMOGLOBIN-SALINE SOLUTION

INTRODUCTION

Baboons consistently show a significant drop in mixed venous PO_2 after exchange transfusion with stroma-free hemoglobin. This is related, at least in part, to the high oxygen affinity of the infused hemoglobin. The change in PO_2 reflects a drop in interstitial and intracellular PO_2, in some organs. The possibility of tissue hypoxia to the heart and brain has been a source of concern to us. Previous work has shown a small but significant loss of cardiac reserve after exchange transfuion. The purpose of this pilot study was to evaluate central nervous system activity during exchange transfusion, using the evoked response as a measure of functional integrity.

MATERIALS AND METHODS

Two male baboons (20-23 kg) were the test animals. They were prepared for exchange transfusion with SFH, using our standard techniques. Somatosensory and visual evoked potentials (SEP and VEP) were obtained from EEG signals using scalp needle electrodes. The recording electrode for the SEP was placed on the scalp over the right post-central gyrus. The recording electrode for the VEP was placed 1 cm to the right of the midline and 2 cm caudal to the posterior occipital protuberance. A reference electrode was placed on the vortex.

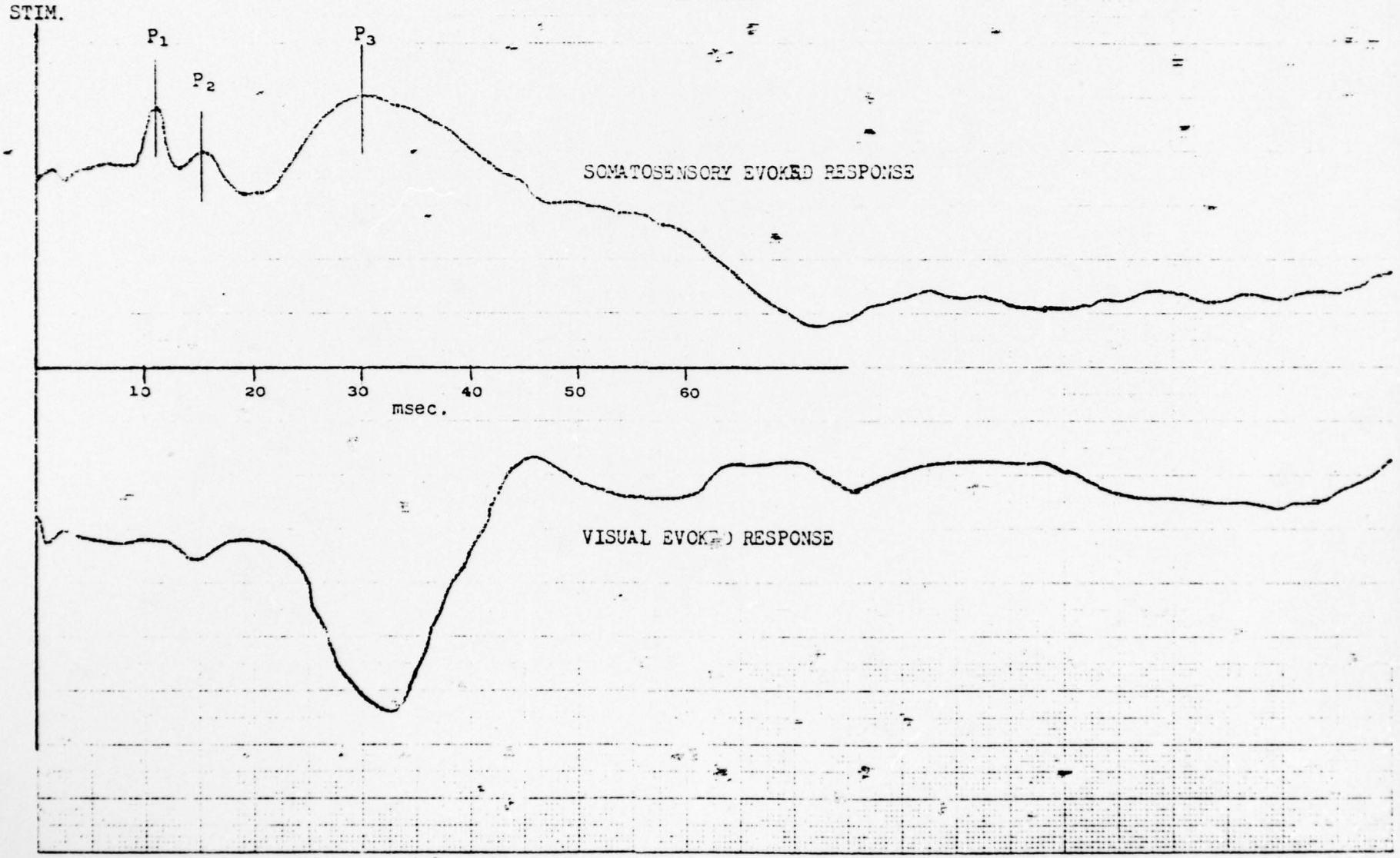
The left median nerve was stimulated for the SEP. The stimulus pulse had a 2 Hz frequency and a 0.1 millisecond duration. The amplitude was set between 40 and 70 volts, which elicited a twitch of the opponens pollicis. The visual stimulus had a frequency of 2 Hz and a flash intensity of 4 R.U. The EEG and stimulus signals were analyzed by a Nicolet Evoked Response Computer. The filter time constant was 1 millisecond; the input sensitivity was $\pm$ 2 volts; and the ADC resolution was 9 bits. The dwell time was 200 milliseconds per address, and an average of 64 sweeps was used. The latency, or elapsed time, from stimuli to each peak of the evoked response, and the peak amplitude of the positive/negative sequence of each evoked response was measured. Increase in latency, and decrease in peak amplitudes are consistent responses of the central and peripheral nervous system to hypoxia.

The evoked potentials were obtained during the control period, and at hematocrits of 30, 20, 10 and 5.

RESULTS AND DISCUSSION

Figure I contains a typical somatosensory and visual evoked response. The times from the onset of stimulus to the first three positive peaks are referred as the P_1, P_2, and P_3 latencies. Table I contains the results for the latencies of the somatosensory evoked responses. No significant trends were apparent during the exchange

FIGURE 1 - EVOKED RESPONSES



transfusion. We observed great variability in peak amplitudes, both in replicate runs from each animal, and between animals. To some extent, this is due to the use of scalp electrodes. The variability should decrease with direct surface electrodes. The small number of animals in this pilot study precludes any statistical analysis of the data.

One result of this pilot study is that both types of evoked response persisted during the total exchange transfusion. The evoked response is a sensitive measure of altered CNS function due to hypoxia. Hence, these preliminary results make it unlikely that massive hypoxia occurs during SFH administration.

TABLE I

Average Latencies of SEP+

HCT	P_1*	P_2*	P_3*
40	11.6 msec.	19.5	33.8
30	13.8	20.3	40.1
20	12.1	14.8	32.4
10	11.8	16.0	42.9
5	12.1	16.0	31.3

* Average from 2 animals

+ Times to first, second and third positive peaks of somatosensory evoked response.

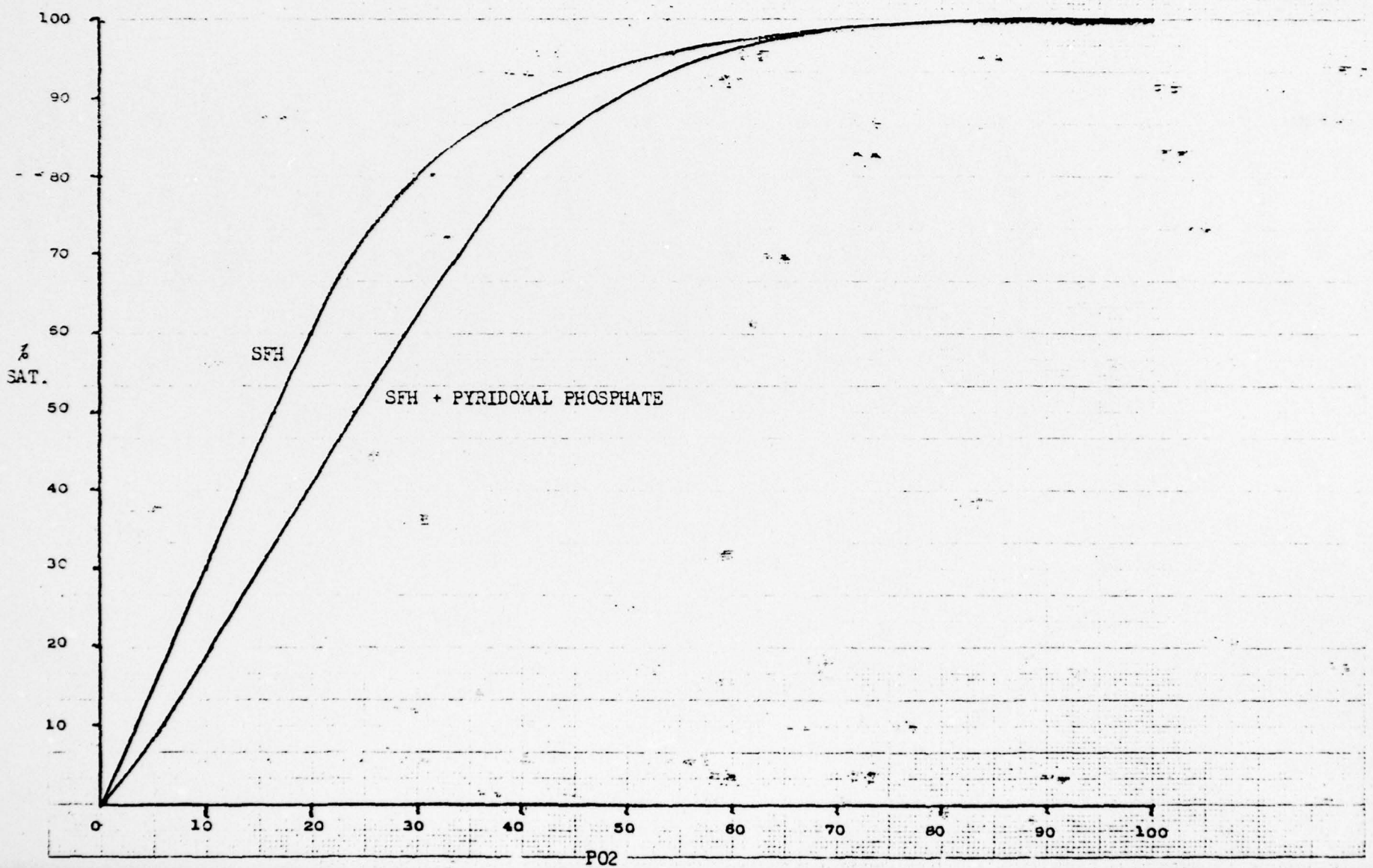
II. WORK IN PROGRESS

1. PERMATIZING OF ORGANIC PHOSPHATES TO HEMOGLOBIN

Organic phosphates containing an aldehyde group can form Schiff bases with the N-terminus of the beta-chain of hemoglobin. If the Schiff base is reduced with sodium borohydride, the organic phosphate becomes covalently bound to the hemoglobin molecule. Pyridoxal phosphate has been bound to hemoglobin, resulting in a molecule with a reduced oxygen affinity. Glycosylated hemoglobin (A_{1c}) can be formed in a similar manner, using glucose-6-phosphate. Several other aldehyde phosphates are under testing for their ability to reduce the oxygen affinity of hemoglobin and their ability to form a Schiff base at the N-terminus of the beta-chain. These compounds include: glycoaldehyde phosphate, glyceraldehyde-3-phosphate, D-erythose-4-phosphate, D-arabinose-5-phosphate, and D-ribose-5-phosphate. Figure 1 contains some oxygen dissociation curves, utilizing the Aminco Heme-O_2-Scan. The left shifted curve was obtained from our SFH. It had a P_{50} of 16 mm Hg and a Hill coefficient of 2.0. The right shifted curve was obtained from a mixture of SFH and pyridoxal phosphate. The P_{50} of this curve was 24 mm Hg and its Hill coefficient was 2.2.

Preparation of small quantities of permatized hemoglobin solution for testing purposes will use the protocol developed by Benesch and Benesch. To 936.4 ml of cold stripped SFH solution (0.27 mM), 63.6 ml of 1 M cold Tris buffer (pH 7.3) is added. Caprylic alcohol (4.6 ml) is added to prevent foaming. The hemoglobin solution is

FIGURE 1 OXYGEN DISSOCIATION CURVES



deoxygenated by bubbling nitrogen through it for 30 minutes. When the solution is devoid of oxygen, 0.55 mM of the organic phosphate in 45.5 ml of Tris buffer is added. The pH of the solution is adjusted to 6.8 with 0.1 N HCl. Nitrogen is bubbled through the solution for an additional 30 minutes with the temperature being maintained at 10° C. 5.5 mM of sodium borohydride in 45.5 ml of 1 M sodium hydroxide is then added to the solution. The hydrolysis of the sodium borohydride will cause the pH to rise to 7.1. Nitrogen is bubbled through the solution for another hour. The reaction at this point is complete. The SFH solution is dialyzed for 2 hours at 5° C against 4 changes of 500 ml of 0.02M phosphate buffer (pH 6.8). The yield from this reaction will be tested for oxygen affinity by processing samples with the Aminco Heme-02-Scan oxygen dissociation analyzer. In vitro stability of the permatized molecule will be determined by use of a closed loop molecule filtration system. Techniques for multi-liter batches of permatized hemoglobin will be developed once an aldehyde phosphate is chosen based on these studies.

Animal Evaluation of Permatized Hemoglobin

The permatized hemoglobin solution (PH) will be compared with the SFH solution reported in Section 1 of work completed. A group of four animals will be exchange transfused to 0 hematocrit with PH.

The animals will be paralyzed and unanesthetized, ventilated on room air, and normothermic. Cardiac output, heart rate, body temperature, arterial pressure, pulmonary and wedge arterial pressures will be monitored. Arterial and mixed venous (pulmonary arterial) samples will be taken for blood gases. Oxygen contents will be measured on whole blood and plasma from arterial and mixed venous samples. Oxygen capacity of whole blood and plasma will be obtained by measuring contents of samples which have equilibrated with air. Oxygen dissociation curves of whole blood and plasma will be determined using the Aminco Heme-O_2-Scan. P_{50} and Hill coefficients for the various hemoglobin compartments will be calculated from these curves. Total hemoglobin, methemoglobin, and sulfhemoglobin concentration of whole blood and plasma will be determined using the Drabkin cyanomet-hemoglobin technique. Effective hemoglobin concentration will be calculated from oxygen capacities. Oxygen consumption will be calculated from cardiac output and A-V O_2 differences. Samples will be obtained at base-line, and at hematocrits of 30, 20, 10, 5 and 0.

At the conclusion of the experiment statistical analysis of the data will be performed to determine whether or not additional animal experiments will be necessary to reach a significant result. This is done to minimize the number of animals used.

2. CEREBRAL RESPONSE TO RED CELL INFUSION AFTER HEMOBLOBIN ADMINISTRATION

Cerebral metabolism and function can be maintained during moderate normovolemic anemia. The primary compensation appears to be an increase in cerebral blood flow. At hematocrits below about 10%, significant alterations occur in the monitored parameters.

Several groups of investigators (1,2,3,4) have reported that after hypotension, ischemia, or severe normovolemic anemia, transfusion of red cells did not reverse defects in observed function or metabolism. We have observed that re-infusion of shed red cells, after the baboon has been at 0 hematocrit and maintained on SFH for serveral hours, results in a significant flattening of the EEG. On autopsy, the brains appear ischemic.

The purpose of this study will be to study the effect of SFH on cerebral metabolism during normovolemic anemia and subsequent tranfusion of shed red cells.

A group of four baboons will be studied. The protocol consists of three stages. Stage 1 is a control period. During Stage 2, the animal is exchange transfused with SFH to some fixed hematocrit and maintained for three hours. In Stage 3, the washed packed shed red cells are transfused, using physiologic pressures. At the end of Stage 3, the animal is sacrificed and the brain removed for examination.

Measurements will be obtained for each stage. These include the standard hemodynamic, blood gas, and content determinations. In addition, the internal jugular vein will be catheterized to obtain mixed venous samples of cerebral blood. Cerebral blood flow will be estimated using the nitrous oxide technique. Oxygen and glucose concentrations will be obtained for cerebral arterial and mixed venous samples. This data will permit us to calculate oxygen and glucose consumption at each stage of the study. A randomized sequence will be employed to assign the Stage 2 hematocrit level for each study. Final hematocrit levels of 20, 10, 5 and 2 will be used.

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3. RENAL EFFECTS OF STROMA-FREE HEMOGLOBIN SOLUTION

METHODS AND MATERIALS

Four adult baboons will be the test animals. One week prior to the study, siliconized heparin-filled polyethylene catheters will be surgically implanted into the left renal vein, and the free end ligated and buried subcutaneiously. After convalescence from surgery, the renal vein catheter will be re-exposed. Polyethylene catheters will be introduced into the aortic arch and suprarenal vena cava via the femoral vessels. A suprapubic cystotomy will provide urinary drainage. The animals will be loosely restrained in the prone position and allowed to recover from tranquilization.

During the four hour baseline period, the animals will receive an intravenous infusion of normal saline with 44 mEq/L of $NaHCO_3$ at a rate of 10 cc/kg/hr. This infusion will insure a constant basal urine output. A primary dose of PAH (40 mg/kg) and Inulin (75 mg/kg) will be given. This will be followed by a constant infusion of 0.5% PAH and 1.0% Inulin in saline, at 6 cc/kg/hr for the remainder of the experiment. At 60 and 75 minutes after priming, arterial and renal vein samples and fifteen minute urine collections will be obtained for the course of the study.

Every 30 minutes thereafter b ood samples will be taken. Beginning with the first 30 minute period, a 50 cc bolus of hemoglobin solution (6 gms %) will be given every 15 minutes.

This will continue until a volume of stroma-free hemoglobin solution (50% of the animals calculated circulating blood volume) has been infused. At the end of this period the animals will be sacrificed and autopsied.

Renal Clearance Determinations: Plasma and urine concentrations of PAH and Inulin will be determined by spectophotometric techniques. The glomerular filtration rate will be determined by:

$$GFR = \frac{(\text{Urinary Conc. of Inulin}) \times (\text{Urine Flow})}{(\text{Plasma Conc. of Inulin})}$$

The renal blood flow will be determined by:

$$\text{Renal Plasma Flow} = \frac{(\text{Urinary Conc. of PAH}) \times (\text{Urine Flow})}{(\text{Renal Art. Conc. of PAH} - \text{Renal Vein Conc. of PAH})}$$

$$\text{Renal Blood Flow} = \frac{\text{Renal Plasma Flow}}{1 - \text{Hematocrit}}$$

The Free Water Clearance will be determined by:

$$FWC = \frac{(\text{Urine Osmolarity}) \times (\text{Urine Volume})}{(\text{Plasma Osmolarity})} - \text{Urine Volume}$$

The urine and plasma osmolarities will be determined by freezing point depression.

Renal A-V Shunting: To test for renal A-V shunting, blood will be drawn anerobically for simultaneous blood gas determinations.

BUDGET

1.	SALARIES	
	A. Biochemist	$ 17,000.00
	Fringe Benefits	1,028.50
	Overhead, 75% of S+W	12,750.00
	B. Biochemistry Technician	$ 13,000.00
	Fringe Benefits	786.50
	Overhead, 75% of S+W	9,750.00
	TOTAL	$ 54,315.00
2.	SUPPLIES	
	A. Baboons - 12 @ $533.50	$ 6,402.00
	B. Animal Care, Approx. $120.00/mo	1,440.00
	C. Surgical Charges	
	$35/surgery x 12 baboons	420.00
	D. Expendable Supplies	700.00
	GRAND TOTAL	$ 63,277.00

BUDGET JUSTIFICATION

PERSONNEL: The salary levels are consistent with the prevailing job market in Chicago. Two people are necessary because of the nature of the studies plus the fact that we will be producing large batches of stroma free hemoglobin solution for designated investigators as well as studying its properties ourselves.

BABOONS: Four (4) for the Permatized Hemoglobin Study, four (4) for the Renal Study, and four (4) for the Cerebral Response Study. Twelve (12) baboons at $533.50 each equals a total of $6,402.00. The average monthly charge for animal care is $120.00 for twelve (12) months, which equals $1,440.00 per year.

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